

- 16 -

- Fig. 2: Identification of inorganic phosphate as reaction product of histidine phosphatase cleavage. The plates show thin layer chromatography (PEI cellulose, 0.8 M LiCl); left panel: ammonium molybdate treatment; right panel: autoradiography treatment; 1: [32 P]his-cheA, 2: reaction product, 3: ATP, 1-3: phosphate (NaH_2PO_4);
- Fig. 3: Time and protein dependent dephosphorylation of the substrate cheA; left panel: degradation after 0, 20 and 40 min, right panel: release of radio labeled phosphate (left y-axis: %, right y-axis: radio activity) of different amounts of substrate (x-axis: ng protein).
- Fig. 4: Analysis of the fraction with active histidine phosphatase (SDS-PAGE); AF: active fraction; 1,2: BSA ($1\mu\text{g}$, $0.5\mu\text{g}$), 3: AF; 4,5: molecular markers.
- Fig. 5: Mass analysis of the histidine phosphatase. Y-axis: counts, x-axis: mass(m/z)
- Fig. 6: Reversed phase chromatographic separation of histidine phosphatase after enzymatic fragmentation; eluent A: 0.1% trifluoroacetic acid in water and eluent B: 20% 0.1% trifluoroacetic acid in water, 80% acetonitrile; UV determination at 214 nm
- Fig. 7a: (partial) Nucleotide sequence of rabbit histidine protein phosphatase
- Fig. 7b: translated complete amino acid sequence of rabbit histidine protein phosphatase
- Fig. 8a: Tumor cell line distribution of histidine protein phosphatase
- Fig. 8b. Tissue distribution of histidine protein phosphatase in tumor tissue.
- Fig. 8c. Tissue distribution of histidine phosphatase in normal tissue.

Patent Claims:

1. A polypeptide having the biological activity of a histidine phosphatase
5 which has a high specificity for phosphohistidine and a molecular weight of 13.000 –15.000, obtainable by purification from mammalian tissue by at least one anion exchange chromatography, one gel filtration and one affinity chromatograph step.
- 10 2. A polypeptide according to claim 1 comprising at least the amino acid sequence motif
DCECLGGGRISHQSQD
- 15 3. A polypeptide according to claim 1 comprising at least the amino acid sequence motif
DCECLGGGRISHQSQDX¹KIHVYGYSMX²YGX³AQH
wherein X¹ = K or R, X² = A or G and X³ = P or R.
- 20 4. A polypeptide according to claim 1 comprising at least the amino acid sequence motif
YHADIYDKVSGDMQKQGCDCECLGGGRISHQSQDKKIHVYGYSM.
- 25 5. A polypeptide having the biological activity of a histidine phosphatase which has a high specificity for phosphohistidine and a molecular weight of 13.000 –15.000, comprising the following amino acid sequence:
(M) AVADLALIPDVIDSDGVFKYVLIRVHSAPRSGAPAAESKEIVRGYKWAIEYH
ADIYDKVSGDMQKQGCDCECLGGGRISHQSQDKKIHVYGYSMAYGPAQHAISTEK
IKAKYPDYEVTWANDGY.
- 30 6. A polypeptide having the biological activity of a histidine phosphatase which has a high specificity for phosphohistidine and a molecular weight of 13.000 –15.000, the amino acid sequence of which has a homology of 64 – 99% compared with the sequence depicted in claim 3.

- 18 -

7. A polypeptide according to claim 6 comprising the following amino acid sequence:

AAAGLAQIPDVDDIDSDGVFKYVLIRVHAAPPSEAPGGESKDIVRGYKWAHEYHADI
YDKVSGELQKKGHDCECLGGGRISHQSQDRKIHVYGYSMGYGRAQHSVSTEKIRA
KYPDYEVTWADDGY.

5

8. A DNA coding for a polypeptide of any of the claims 1 – 7.

9. A DNA according to claim 8 comprising the nucleotide sequence:

(ATG) GCGGTGGCGGACCTCGCTCTCATTCCTGATGTGGACATCGACTCCGACGG
CGTCTTCAAGTATGTGCTGATCCGAGTCCACTCGGCTCCCCGCTCCGGGGCTCCG
GCTGCAGAGAGCAAGGAGATCGTGCGGGCTACAAGTGGGCTGAGTACCATGCGG
ACATCTACGACAAAGTGTCGGGCGACATGCAGAAGCAAGGCTGCGACTGTGAGTG
TCTGGGCGGCGGGCGCATCTCCCACCAGAGTCAGGACAAGAAGATTCACGTGTAC
GGCTATTCCATGGCCTATGGTCCTGCCAGCACGCCATTTCAACTGAGAAAATCA
AAGCCAAGTACCCCGACTACGAGGTCACCTGGGCTAACGACGGCTAC

10

15

10. Pharmaceutical preparation comprising a polypeptide according to any of the claims 1- 7, where appropriate, together with suitable excipients, carriers and other active ingredients.

20

11. An antibody directed to a polypeptide according to claims 1- 7.